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Effects of Subchronic Exposures to Concentrated Ambient Particles (CAPs) in Mice: III. Acute and Chronic Effects of CAPs on Heart Rate, Heart-Rate Fluctuation, and Body Temperature

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Normal mice (C57) and mice prone to develop atherosclerosis (ApoE$^{-/-}$) were implanted with electrocardiograph (EKG), core body temperature, and motion transmitters and were exposed daily for 6 h to Tuxedo, NY, concentrated ambient particles (CAPs) for 5 days/wk during the spring and summer of 2003. Time series of 5-min EKG monitoring and body-temperature measurements were obtained for each animal in the CAPs and filtered air sham exposure groups. Our hypothesis was that chronic exposure could cause cumulative health effects. We used our recently developed nonparametric method to estimate the daily time periods that mean heart rates (HR), body temperature, and physical activity differed significantly between the CAPs and sham exposed groups. CAPs exposure most affected heart rate between 1:30 a.m. and 4:30 a.m. With the response variables being the average heart rate, body temperature, and physical activity, we adopted a two-stage modeling approach to obtain the estimates of chronic and acute effects on the changes of these three response variables. In the first stage, a time-varying model estimated daily crude effects. In the second stage, the true means of the estimated crude effects were modeled with a polynomial function of time for chronic effects, a linear term of daily CAPs exposure concentrations for acute effects, and a random component for unknown noise. A Bayesian framework combined these two stages. There were significant decreasing patterns of HR, body temperature, and physical activity for the ApoE$^{-/-}$ mice over the 5 mo of CAPs exposure, with smaller and nonsignificant changes for the C57 mice. The chronic effect changes of the three response variables for ApoE$^{-/-}$ mice were maximal in the last few weeks. There was also a significant relationship between CAPs exposure concentration and short-term change of heart rate in ApoE$^{-/-}$ mice during exposure. Response variables were also defined for examining fluctuations of 5-min heart rates within long (i.e., 3–6 h) and short time periods (i.e., $\sim$15 min). The results for the ApoE$^{-/-}$ mice showed that heart-rate fluctuation within the longer periods increased to 1.35-fold by the end of exposure experiment, while the heart-rate fluctuation within 15 min decreased to 0.7-fold.

A key requirement for the overall study described by Lippmann et al. (2005) of the effects of inhaling concentrated ambient particles (CAPs) on the health of normal (C57) and a murine model for humans with aortic plaque (ApoE$^{-/-}$) mice during the course of subchronic exposure study is an unbiased method for identifying and characterizing abnormal cardiac function. The experimental plan involves continuous electrocardiographic (EKG) monitoring of the heart and body core temperature by an implanted monitor before and during 5 mo of daily weekday exposures of 6 h duration. The physical activity was also recorded. This experiment has generated an enormous volume of electronic data, even with the decision to record only 5 min of each hour. One of the main hypotheses of this study is

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that subchronic exposure of normal and compromised mice to CAPs will cause cumulative adverse effects on the cardiovascular systems and locomotive behavior. However, it was not clear, at the outset, what the temporal lags for effects of the exposures on the scales of hours, days, or months might be. It was also important to avoid subjective evaluation techniques that could not stand up to rigorous inquiry. These made it even more difficult to characterize the effect and test the hypotheses.

Some of the same problems were faced in conducting previous shorter term CAPs exposure studies in rats in this laboratory (Nadziejko et al., 2003). Since there were no established methods for statistical analysis of telemetry monitoring data in rodents, Nadziejko et al. (2004) developed a new method for analyzing the time course of effects in repeated measurements and applied it to the short-term CAPs exposure data. This nonparametric method, called the "fishing license" method, is based on (1) a test statistic of the largest absolute value of *t*-statistic between two groups obtained by considering all possible time intervals in the observed time period and (2) a bootstrap null distribution of the test statistic for determining a critical value. The method also estimates optimal onset and ending times. Once the time period was determined, we could define response variables as the daily averaged measurements over the selected time period of the day for each mouse to test the differences between exposure and control groups.

To estimate both chronic effect and acute effect simultaneously and simplify the computations from the very large data set of the subchronic CAPs inhalation study in mice, we proposed a novel Bayesian two-stage modeling approach. This approach made it possible to analyze the data for evidence of (1) acute effects of daily peaks of exposure on cardiac function, activity and body temperature during the exposure day and (2) changes in baseline cardiac function, activity, and body temperature during the course of the subchronic series of exposures.

EXPERIMENTAL DESIGN AND DATA

This study is part of a subchronic CAPs exposure study investigating the effects of CAPs on cardiovascular system. The rationale and experimental design of this study is detailed elsewhere in this special issue (Lippmann et al., 2005). In this study, the normal mice (C57) and knockout transgenic mice lacking apolipoprotein E ($ApoE^{-/-}$) were randomly assigned into control and exposure groups in the subchronic study. C57 mice were obtained from the Jackson Laboratory (Bar Habor, ME) and $ApoE^{-/-}$ mice were obtained from Taconic Europe (Demark). The mice with implanted transmitters were housed singly in our ALAAC-accredited animal housing facility at Tuxedo so that the EKG parameters were monitored continuously. Starting at 7 months before the start of the CAPs exposures, $ApoE^{-/-}$ mice were fed with a high-fat diet (Adjust Calories Diet, TD88137, Harlan, Indianapolis, IN) for 4 mo. Severe skin irritation developed in some of these mice, and all $ApoE^{-/-}$ mice were switched to a normal diet 3 mo prior to the CAPs exposures. The C57 mice were on a regular diet throughout, and had access to food and water ad libitum. The light/dark cycle was to be maintained throughout the study through the use of an automatic room light switch, but the controller failed, and the room light remained on all day for the period from late June to early July until it was remedied.

Animals were exposed to northeastern regional background CAPs using a modified VACES system developed by Sioutas et al. (1999). The detailed design and performance of the entire system as well as exposure atmosphere characterization are described elsewhere in this special issue of *Inhalation Toxicology* (Maciejczyk et al., 2005). Briefly, the exposures were conducted in our Sterling Forest laboratory in Tuxedo, NY, 40 miles northwest of Manhattan. Animals were exposed to filtered air or CAPs at 10× of ambient concentrations for 6 h/day, 5 d/wk. The exposures started on April 11, 2003, and ended on September 5, 2003.

Monitoring continued for five nonexposure days to September 10, 2003. The animals with implanted EKG telemeters were exposed to either filtered air (control group) or air and CAPs (exposure group) around 9:00–15:00 on weekdays and stayed in an animal room with filtered air for the rest of the time, except for short time periods required to move in and out of the exposure chambers. Ten-second averages of heart rate and body temperature were obtained every 5 min. Activity counts for every 5-min interval were expressed as counts per minute. There were 9 $ApoE^{-/-}$ mice in the control group and 10 in the exposure group. Although the experiment started with six C57 mice in both groups, due to battery and some mechanical problems, complete data for three mice in this exposure group were not available for analysis. The average of CAPs mass concentrations during each day's 6-h exposure was calculated for use in examining any acute concentration-response effects. The light in the animal room was turned on at 6 a.m. and off at 6 p.m. automatically to control the living environment consistent over the 5 mo of experiment. Due to some undetected mechanical problems, the light was on 24 h/day during late June and early July, and this event may have had a large impact on heart rate, physical activity, and body temperature measurements for that time interval and possibly beyond.

STATISTICAL METHODS

Analysis of Acute and Cumulative Effects of CAPs on Physiological Parameters

The nonparametric method that we developed for testing whether the means of two repeatedly measured groups are significantly different over some unknown interval within a fixed longer interval and for identifying a time interval (or intervals) where the group means are significantly different is described in detail elsewhere (Nadziejko et al., 2004). To test any cumulative effect on circadian pattern changes, we applied this nonparametric method directly to time series of 5-min measurements of mice in the exposure and control groups over the last 5 nonexposure days (9/6–9/10/2003) for $ApoE^{-/-}$ and C57 mice, separately,

to obtain the most significant time periods at which cumulative CAPs exposure effects may exist.

Let y_{ijtk} be the kth 5-min measurement starting from 9:00 a.m. of the tth day for the jth animal in the ith mouse category. Let the identified or given time period begin at the k_1th and end at the k_2th 5 min. The response variable is defined as averaged measurements over the selected time period of the day, that is, $y_{ijt} = \sum_{k_1}^{k_2} y_{ijtk}/(k_2 - k_1 + 1)$. A two-stage model is used to fit the observed values of the response variable. In the first stage, we estimate crude effects of CAPs on C57 and ApoE$^{-/-}$ mice on each day from a time-varying regression model (Shumway & Stoffer, 2000). The time-varying model is given by:

$$\begin{aligned} y_{ijt} &= \gamma_{0t} + \gamma_{1t} I(i \in \text{ApoE}) + \gamma_{2t} I(j \in \text{PM}) \\ &\quad + \gamma_{3t} I(i \in \text{ApoE and } j \in \text{PM}) \\ &\quad + \sum_{l=1}^{t-h} \varphi_{tl}(y_{ij,t-l} - \hat{y}_{ij,t-l}) + e_{ijt} \end{aligned} \quad [1]$$

for $t = 1, 2, 3, \ldots, n$, where $\text{Var}(e_{ijt}) = \sigma_t^2$, $I(\cdot)$ is an indicator function, and h is a fixed number to be determined by Akaike information criterion (AIC) (Akaike, 1973). The coefficients φ_{tl} are included for adjusting possible biases due to animal to animal difference. The predicted value of $y_{ij,t-l}$, $\hat{y}_{ij,t-l}$, is obtained from the previous lth model. To estimate the parameters, we actually do it step by step. Start with $t = 1$ and estimate the parameters and obtain $\hat{y}_{ij1}$ for all mice. Then move on $t = 2$ and obtain $\hat{y}_{ij2}$. Repeat the procedures to $t = n$, the last day. The estimates of crude effects on changes of these variables for C57 and ApoE$^{-/-}$ mice on the tth day are $\hat{\gamma}_{2t}$ and $\hat{\gamma}_{2t} + \hat{\gamma}_{3t}$, respectively.

In stage two of the Bayesian hierarchical model, we assume that the estimated crude effect z_t, either $\hat{\gamma}_{2t}$ or $\hat{\gamma}_{2t} + \hat{\gamma}_{3t}$, is normally distributed with unknown mean θ_t and known standard deviation $\hat{\sigma}_t$, which is the standard error of z_t obtained in stage one. The daily effect parameters $\theta_1, \theta_2, \ldots, \theta_n$ are further modeled by a polynomial function of time representing chronic effect change, a linear term of CAPs exposure mass concentrations for exposure day, a dummy variable corresponding to days affected by the wrongly set light system, and a random component for model uncertainty. Specifically,

$$\begin{aligned} \theta_t &= \sum_{k=0}^{p} \alpha_k \times \max(0, t - \omega)^k + (\beta_0 + \beta_1 X_t) \times I_t + \phi \times L_t + \varepsilon_t \\ &= \mu_t(\alpha_0, \alpha_1, \ldots, \alpha_p, \omega, \beta_0, \beta_1, \phi) + \varepsilon_t \end{aligned} \quad [2]$$

where I_t is an indicator function of exposure day, X_t is a log transform of CAPs exposure concentration, L_t is another indicator function for days in the time interval June 28–July 20, and ε_t is normally distributed with mean 0 and variance τ^2. In the model, we assume the onset of chronic effect is ω days from the start of the experiment. Before onset, the mean effect difference between the exposure and control groups is a constant α_0, which is interpreted as an animal's baseline difference between the two groups. After onset, the chronic effect function is determined by the order p of the polynomial function, which may be determined by the Bayesian deviance information criterion (DIC), which is viewed as a Bayesian analogue of AIC (Spiegelhalter et al., 2002). The parameter β_1 describes the relationship between daily exposure mass concentration and acute effect change. If β_1 is not significantly from 0, β_0 itself can be interpreted as the effect of exposure day against nonexposure day. The parameter ϕ is used for adjusting different impacts on mice in exposure and control groups of the room light system failure that went undetected for several weeks during the exposure period.

We further assume that these parameters in the second stage model are random. Noninformative priors are given for each parameter. The parameters $\alpha_0, \alpha_1, \ldots, \alpha_p$, β_0, β_1, and ϕ are assumed normal distributions, with mean 0 and a large variance of 10,000. The time location parameter ω is uniformly distributed between 0 and n. The prior of the model uncertainty parameter τ^2 is assumed inverse gamma with hyperparameters $b_1 = 2.001$ and b_2 equal to the inverse of sample variance of z_t so that prior mean is close to variation of these z_t and prior variance is around 1000.

The model proposed in this stage is similar to the indirect observational model (Liu, 2001). Let $\Delta = (\alpha_0, \alpha_1, \ldots, \alpha_p, \omega, \beta_0, \beta_1, \phi, \tau)$ be a parameter vector in some parameter space and $\Theta = (\theta_1, \theta_2, \ldots, \theta_n)$ be a random vector whose distribution is normal if Δ is given. However, we cannot directly observe Θ, but observe, instead, the vector $Z = (z_1, z_2, \ldots, z_n)$ and $V = (\hat{\sigma}_1, \hat{\sigma}_2, \ldots, \hat{\sigma}_n)$ whose relation can be described as:

$$\begin{aligned} z_t &= \theta_t + e_t, e_t \sim N\left(0, \hat{\sigma}_t^2\right) \\ \theta_t &= \mu_t(\alpha_0, \alpha_1, \ldots, \alpha_p, \omega, \beta_0, \beta_1, \phi) + \varepsilon_t, \varepsilon_t \sim N(0, \tau^2) \end{aligned}$$

Of interest is the Bayesian inference on the parameter vector and functions of elements in Δ. Let the joint prior distribution $\pi(\Delta)$ be the product of priors of these hyperparameters. The joint posterior of Δ and Θ can be derived as follows:

$$\begin{aligned} \pi(\Delta, \Theta \mid Z, V) \propto \prod_{t=1}^{n} [& f(z_t \mid \theta_t, \hat{\sigma}_t) \\ & \cdot f(\theta_t \mid \mu_t(\alpha_0, \alpha_1, \ldots, \alpha_p, \omega, \beta_0, \beta_1, \phi), \tau) \cdot \pi(\Delta)] \end{aligned}$$

where $f(\cdot \mid a, b)$ stands for a density function of normal distribution with mean a and standard deviation b. The posterior distribution of $\pi(\Delta \mid Z, V)$ is then a marginal of $\pi(\Delta, \Theta \mid Z, V)$. Posterior samples of Δ and Θ can be generated from the target distribution $\pi(\Delta, \Theta \mid Z, V)$ using a free package WinBUGS (WinBUGS Project, http://www.mrc-bsu.cam.ac.uk/bugs, 2004). The sample mean of the simulated posterior samples for a parameter is called the posterior mean of the parameter. The interval between the 2.5th and 97.5th percentiles of the posterior samples of a parameter is called a 95% equal-tail credible interval (CI) of the parameter. For each posterior sample set $(\alpha_0, \alpha_1, \ldots, \alpha_p, \omega)$, we can calculate a posterior curve of chronic effects, which is $g(t) = \sum_{k=0}^{p} \alpha_k \times \max(0, t - \omega)^k$.

With thousands of posterior curves of chronic effects, we calculate posterior means and 95% equal-tail credible intervals for each time point. To estimate the change of the chronic effects between the last day and the first day of the experiment, we may simply define a new parameter $\delta = g(n) - g(1)$. The posterior mean and 95% credible interval of δ can be obtained from the differences between posterior samples of $g(n)$ and$g(1)$.

Heart-Rate Fluctuation Analysis

A fast Fourier transform with Daniell smoother, available in S-Plus, was used to estimate power spectral densities for all the 5-min heart-rate measurements collected on weekends and for the last 5 days of measurements. The nonparametric method was applied to test whether there is an unspecified frequency band during which the mean $\log_{10}$ transformed powers were significantly different between exposure and control groups during the last 5 nonexposure days. The response variable for heart-rate fluctuation is defined as the averaged $\log_{10}$ transformed power over the identified frequency band for each weekend. A similar two-stage model was constructed to estimate the ratios of heart rate fluctuations between exposure and control groups over the 22 weekends within the exposure interval. In the first stage, the group differences of $\log_{10}$ powers are calculated for each weekend. These group differences are called crude effect estimates of heart-rate fluctuation, denoted by z_t for the tth weekend. The standard error of z_t is estimated using the pooled sample standard deviation and denoted by $\hat{\sigma}_t$. In the second stage models, we have $z_t = \theta_t + e_t$, $e_t \sim N(0,\ \hat{\sigma}_t^2)$, $t = 1, 2, \ldots, 22$ and $\theta_t = \sum_{k=0}^{p} \alpha_k \times \max(0,\ t - \omega)^k + \varepsilon_t$, where $\varepsilon_t \sim N(0,\ \tau^2)$. The true chronic effect change is modeled by another polynomial function. The same noninformative priors are given for each parameter. The posterior curves of μ_t are generated through the same procedures already described.

RESULTS

The daily series of 6-h CAPs mass concentrations measured on exposure days is given in Figure 1. It is clear that higher concentrations occurred in the summer than in the spring. The series of concentrations fluctuated over the days with a range from 5 to 627 μg/m^3. The mean CAPs concentration was 133 μg/m^3, and there was a narrow interquartile range of 52–153 μg/m^3. The volume size distribution (particle size) was calculated for 4 daily samples during the exposure period and the mean value of the 4 daily medians was 389 $\pm$ 2 nm (daily median $\pm$ SD). The mean concentrations of ozone and nitrogen dioxide in the CAPs exposure chamber were 10 ppb and 4.4 ppb, respectively.

To select a time period of the day at which the cumulative measurements between exposure and control groups have the most significant difference, we examined the measurements recorded in the last 5 nonexposure days. Figure 2 shows the mean circadian body temperatures with 2 standard errors for the two groups of ApoE$^{-/-}$ mice. The plot indicates that the two groups have different means in the early morning. Our proposed

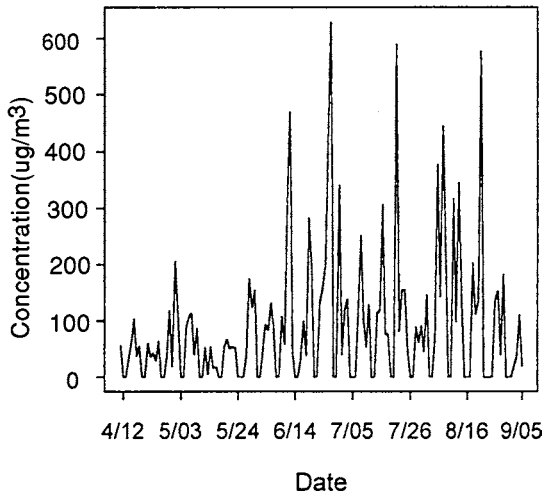

FIG. 1. Time series of daily exposed CAPs concentrations over the 5 mo of experiment. The concentration is given as 0 for nonexposure day.

nonparametric method identified the time period of 1:30 a.m.–4:30 a.m. at which the 2 group means were significantly different. For the HR measurements, we found the two groups had the largest cumulative difference around this time period as well, although the difference was only marginally significant based on the nonparametric method. Therefore, we first defined a daily response variable as the average of 5-min measurements over 1:30 a.m.–4:30 a.m. on the next day for exploring the chronic and acute effects. The proposed Bayesian two-stage models were then applied to fit values of the response variables for heart rate, activity, and body temperature of ApoE$^{-/-}$ and C57 mice collected during the 153 days of the chronic exposure series.

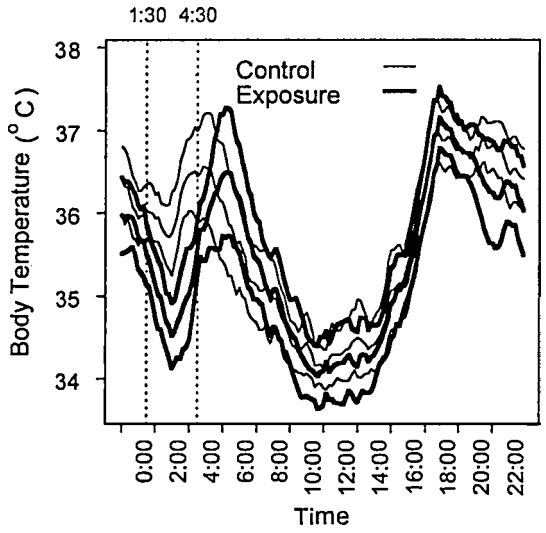

FIG. 2. The group mean 24-h body temperatures with $\pm$2 standard errors of ApoE$^{-/-}$ mice in CAPs exposure and control groups averaged over the last 5 days of the experiment.

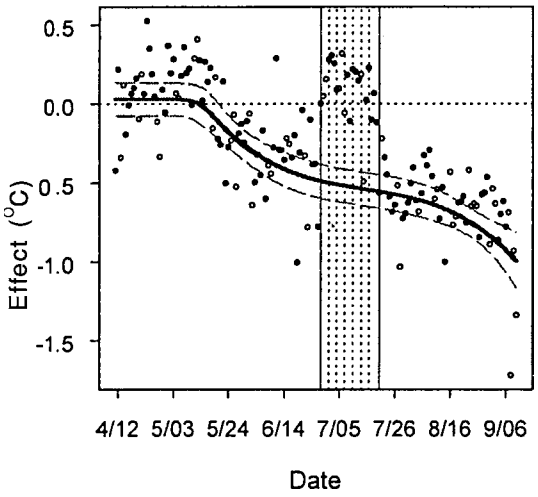

FIG. 3. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for ApoE$^{-/-}$ mice body temperature during 1:30–4:30 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

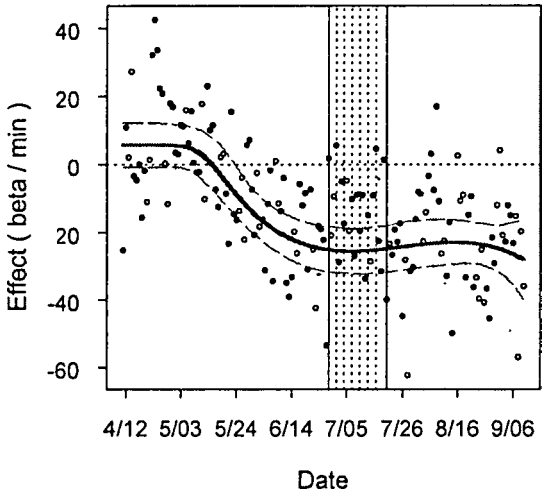

FIG. 5. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for ApoE$^{-/-}$ mice heart rate during 1:30–4:30 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

The crude effects of HR, physical activity, and body temperature estimated from the first-stage models for ApoE$^{-/-}$ mice are illustrated in Figures 3–5. The full circles are for crude effect estimates on exposure days, while empty circles are for nonexposure (mainly weekend and holiday) days. For each case, we computed the deviance information criterion (DIC) for different orders of the polynomial function and chose the order with smallest DIC as the final model. In the case of ApoE$^{-/-}$ mice's body temperature, the two groups were not significantly different in the first 33 days. The chronic effect changes were best modeled by a cubic polynomial function. Figure 3 shows the posterior means of the chronic effect changes over days with 95% credible intervals. It is very clear that the chronic CAPs exposure was associated with a decreasing body temperature. Figure 4 shows that the activity was smaller at the beginning for ApoE$^{-/-}$ mice in the exposure group. The estimated chronic effects of CAPs on activity started decreasing linearly right from the beginning, and continued to the end of the exposure period. The chronic effects of CAPs on HR were modeled by a cubic polynomial function. The estimated posterior mean and 95% CI of the chronic effect changes are shown in Figure 5. The cumulative effects of CAPs on HR appeared after about 30 days as a decreasing heart rate.

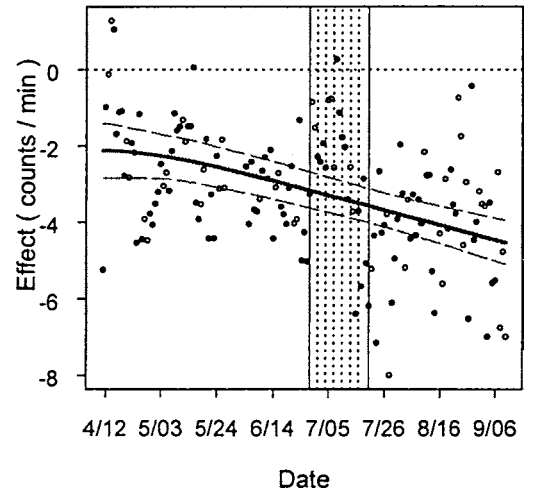

FIG. 4. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for ApoE$^{-/-}$ mice activity during 1:30–4:30 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

The estimated chronic effects changes between the last day and the first day of the 5-mo experiment for the 3 response variables are summarized as follows: The body temperature dropped about 1.0°C with 95% CI (0.8, 1.2). The activity had a decrease of 2.4 counts per minute with 95% CI (1.5, 3.3). The HR dropped about 33.8 beats per minute (bpm) with 95% CI (20.9, 46.3). The amount of decrease is about 6% of the average heart rate of nonexposed ApoE$^{-/-}$ mice. We found no association with daily CAPs concentration and no difference of exposure against nonexposure days for this time period (1:30–4:30). It can also be seen that the impact of the animal room light system failure

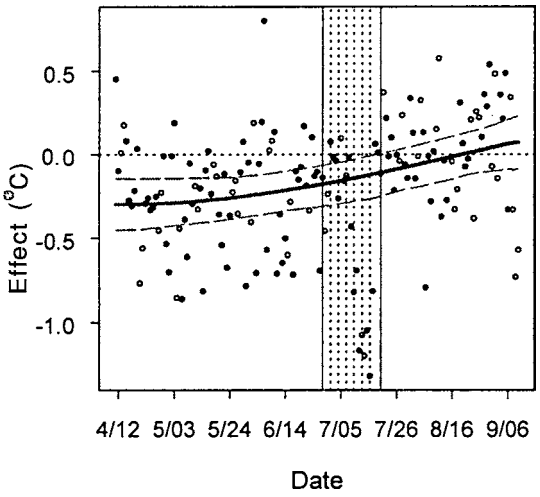

FIG. 6. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for C57 mice body temperature during 1:30–4:30 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

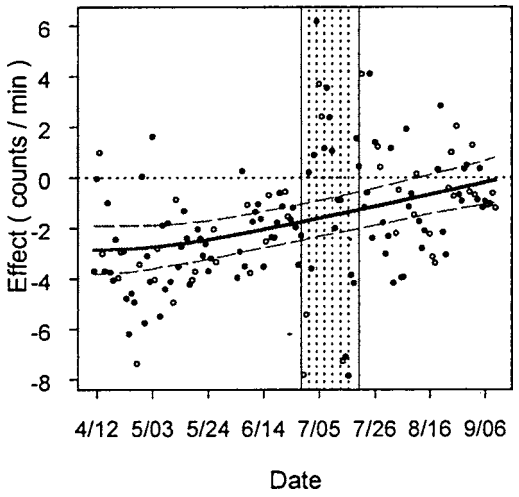

FIG. 7. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for C57 mice activity during 1:30–4:30 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

had a positive effect adjustment of 0.53°C on body-temperature differences with 95% CI (0.4, 0.66). The model estimates also showed the activity differences between exposure and control mice were reduced during the light system failure period with a mean adjustment of 1.5 counts/min and 95% CI (0.7, 2.2). However, the adjustment on heart rate was marginal with mean 7.5 bpm and 95% CI (−0.1, 15).

For the case of the C57 mice at this daily time period, we found that linear polynomial functions fit best for the chronic effect changes of body temperature, activity, and heart rate, respectively. Figure 6 shows the chronic effect changes of C57 mice's body temperature. The posterior mean body temperature of the three mice in the CAPs exposure group was lower than for the six mice in the control group at the beginning and then gradually increased. By the end of experiment, there was a mean difference of 0.4°C between the exposure and control groups. The light system failure had adjusted body temperature back by 0.19°C. Figure 7 shows that chronic effects on activity in the C57 mice were very similar to those on body temperature. The chronic effects for the C57 mice's HR are shown in Figure 8, indicating a constant higher mean HR in the exposure group, with a slight increase near the end of exposure series.

Figure 2 shows that mice had the lowest body temperatures during the time period of 11:00–13:00. The mice were also in the exposure chamber during this time period on exposure days. Therefore, we were interested in examining effects during this time period, especially for the ApoE$^{-/-}$ mice. We defined new response variables of daily heart rate, activity, and body temperature as measurements averaged over the time period of 11:00–13:00. The crude effects estimated from the first-stage model are plotted in Figures 9–11 for ApoE$^{-/-}$ mice body temperature, activity, and HR. Linear polynomial functions were best fit for chronic effect changes of body temperature, activity, and HR during this time period. The posterior mean curve of the chronic effect changes shown in Figure 9 indicates that

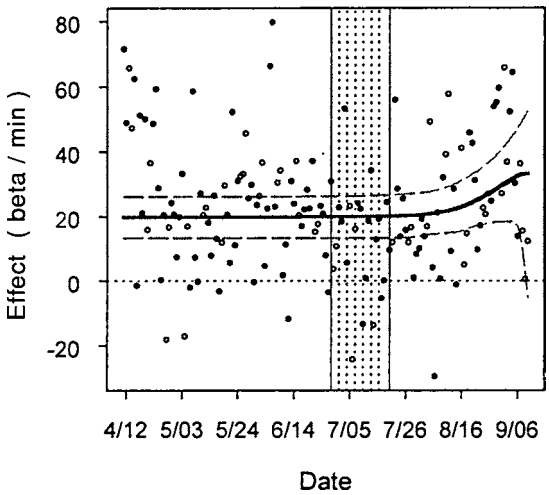

FIG. 8. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for C57 mice heart rate during 1:30–4:30 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

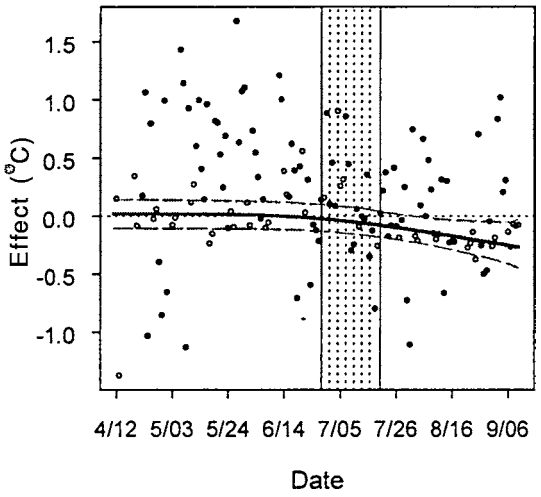

FIG. 9. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for ApoE$^{-/-}$ mice body temperature during 11:00–13:00 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

body temperature started decreasing at about 50 days, and had a marginally significant drop of 0.29°C by the end of the exposure series. Figure 10 shows that the chronic effect change of activity pattern was similar to that for body temperature with a significant drop of 0.5 counts/min. The chronic effect of CAPs on HR

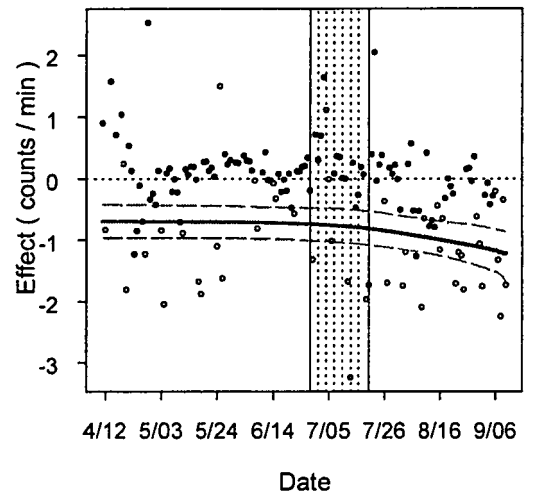

FIG. 10. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for ApoE$^{-/-}$ mice activity during 11:00–13:00 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

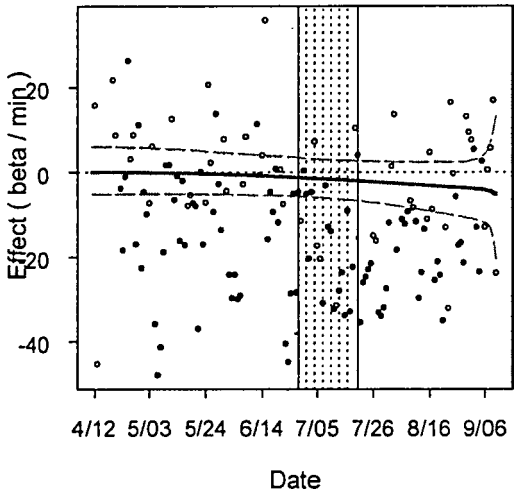

FIG. 11. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes for ApoE$^{-/-}$ mice heart rate during 11:00–13:00 obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage (full for exposure day and empty for nonexposure day). The light failure dates are marked with vertical bars.

(Figure 11) exhibited a slightly decreasing pattern, but no significant change from the beginning to the end. In Figures 9 and 10 the chronic effect curves for body temperature and activity appear to be much lower, while in Figure 11 for HR, the curves are much higher than the full circles of estimated crude effects on exposure days. These results indicate that, during exposure, acute effects exist for body temperature, activity and heart rate changes. We found that body temperature increased by an average of 0.64°C, with a 95% CI (−0.01, 1.28) during the exposure period and no association between CAPs level and body temperature change. The activity increased by 0.9 counts per minute 95% CI (0.3, 1.5) and had no relationships with CAPs concentration. However, HR change during the exposure period was marginally associated with the CAPs level. The posterior mean (95% CI) of the slope parameter for log CAPs level, β_1, is −2.54 (−5.33, 0.16). That is, the HR of ApoE$^{-/-}$ mice decreased by an average of 12.4 bpm for the mean exposed CAPs concentration of 133 μg/m^3. Whether the acute effects of increased activity and body temperature were directly caused by the CAPs exposure needs further investigation. We have found that the mean temperature in the CAPs exposure chamber was 0.4°C lower than that in the filtered air exposure chamber. The cross-correlations among HR, activity, and body temperature during 9:00–14:00 were much lower on exposure days when the mice were confined in the small dark cells than on weekends. The chamber effect may be part of the cause for the acute effects of CAPs on activity and body temperature.

Finally, we further investigated fluctuations of heart-rate series between exposure and control groups. The estimated power spectral densities for the 5-min heart-rate measurements in the

mice collected on the last 5 days were first calculated. The nonparametric method identified a low-frequency band (0.0125–0.0229), during which the cumulative powers of the two groups were significantly different (significance level $<.05$), and a high-frequency band 0.3–0.3146 during which the cumulative powers of the 2 groups were marginally different (significance level $<.07$). The low frequency band corresponds to 3.6–6.7 h. The high-frequency band corresponds to 16–17 min. Two response variables were defined as the averaged $\log_{10}$ estimated powers over the 2 frequency bands transformed from the 5-min heart rates during each weekend. The group differences of the two response variables over the 22 weekends are plotted in Figures 12 and 13. Based on DIC values, linear polynomial functions were determined for both low- and high-frequency response variables. The posterior mean differences of $\log_{10}$ power for the low-frequency band between exposure and control groups, shown in Figure 12, show little change in the first 15 wk, but were clearly increasing in the last 7 wk. For the high-frequency band shown in Figure 13, we found the same pattern, but of opposite direction. The posterior mean with 95% credible interval of chronic effect change of spectrum power difference between the last and the first weekend were 0.13 with 95% CI (-0.04, 0.32) and -0.16 with 95% CI (-0.31, -0.01) in low- and high-frequency bands, respectively. Based on the spectral analysis of 5-min heart rate series, we conclude that fluctuation of the 5-min heart-rate series within longer time intervals increased by 1.35-fold by the end of exposure experiment, while the fluctuation of the 5-min heart rates within short-term intervals ($\sim$15 min) decreased by 0.7-fold.

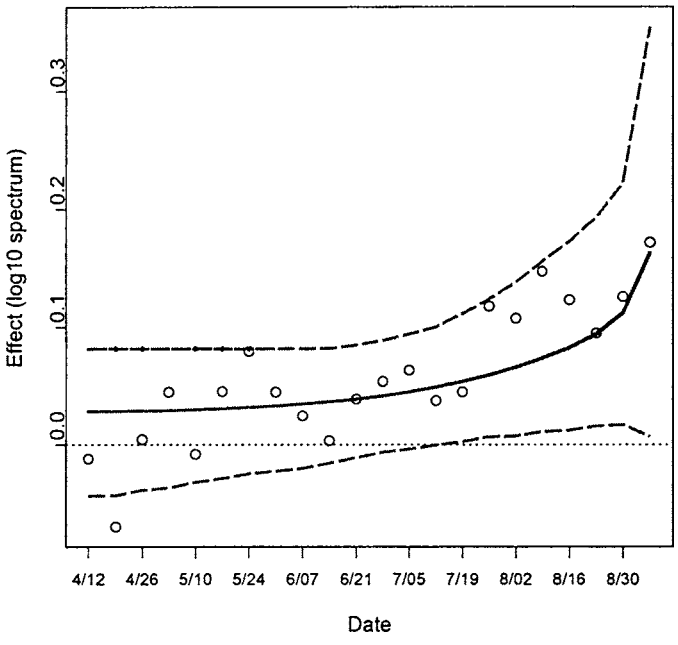

FIG. 12. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes of $\log_{10}$ spectrum powers over low-frequency band 0.0125–0.0229 for ApoE$^{-/-}$ mice heart rate obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage.

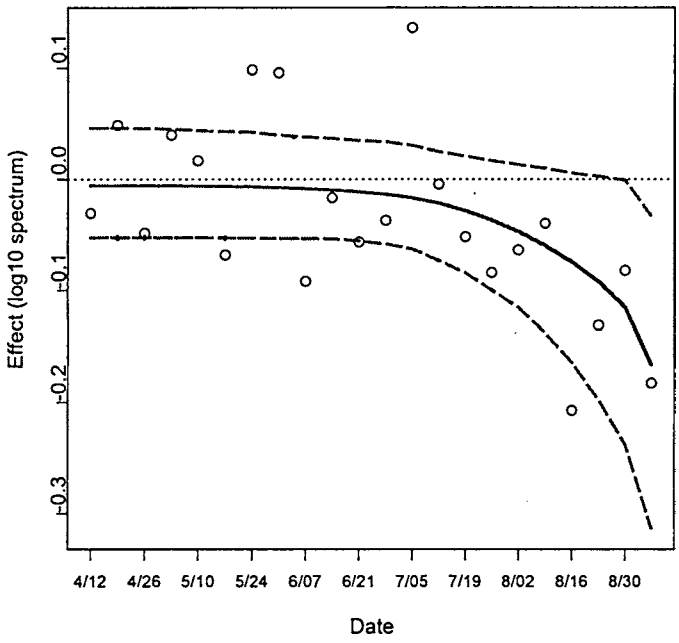

FIG. 13. The posterior means (solid) and 95% equal-tail credible intervals (dotted) of chronic effect changes of $\log_{10}$ spectrum powers over high-frequency band 0.3–0.3146 for ApoE$^{-/-}$ mice heart rate obtained from the Bayesian model in the second stage. The circles in the plots are daily crude effects estimated in the first stage.

CONCLUSIONS

While the subchronic animal inhalation was expected to produce a huge amount of measurement data, no clear guidelines existed, prior to this study, for characterizing the CAPs exposure effects. Also, no available classic statistical models could be directly used to fit these complex multiple time-series. These made the study quite challenging. To make the analysis feasible, and to extract as much information as possible from the data, we proceeded in three steps. First, we applied our previously developed nonparametric method to identify the time period (1:30–4:30 a.m.) during which CAPs exposure had the largest effect each day. Second, we defined a response variable as the averaged measurements over the selected time period to generate daily series sufficient for chronic effects examination. Third, we applied two-stage Bayesian models to find the chronic effect changes. Bayesian approaches have been widely applied to model practical data, since generating posterior samples is feasible via Monte Carlo simulations. Using the free package WinBUGS, our computations were feasible to implement, and CPU times were not a problem.

Our analysis of subchronic CAPs exposure data has enabled the study team to demonstrate that the CAPs exposures at Tuxedo, NY, during the 5 warmer months of 2003 that averaged 133 $\mu g/m^3$ during the 30 h/wk of exposure produced significant responses in a susceptible animal model, in terms of both acute and cumulative changes in heart rate, activity, and body core temperature. It is notable that the effects were seen for exposures that did not greatly exceed either the current 24-h or annual average National Ambient Air Quality Standard for fine particulate matter. Our analysis procedures were also applied to examine fluctuations of 5-min heart-rate series. The interpretation of the findings has to be cautious, because this index differs from the traditional heart rate variability (HRV) analysis of RR intervals. A separate analysis of the effects of the subchronic CAPs exposure on HRV in the mice is presented by Chen and Hwang (2005) in this special issue of *Inhalation Toxicology*.

This method provides a powerful analysis tool for future toxicological studies, and may also be applicable for human panel studies of individuals with chronic cardiac diseases who are willing to wear continuous cardiac monitors.

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